CURRENT ASPECTS OF CANCER RESEARCH

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THROUGH basic research, progress in the attack on cancer has been remarkably rapid in the past several years and continues at an encouraging and accelerating pace. It is the goal of this presentation to appraise some of the recent experimental developments which give us particular cause for optimism concerning the ultimate solution of the cancer problem.

Experimental Cancer Systems

A major factor in the understanding of medical problems in the past has been the ability to study the disease entity under controlled conditions. From such research have come the revolutionary advances in medical science: the vitamins, hormones, and antibiotics currently in use. Concerning this aspect of the cancer problem we are presently in an unusually favorable position, as we can now reproduce the disease easily under laboratory conditions. Table 1 lists certain examples from a variety of agents which will induce cancer with a high degree of predictability. Practically any type of tissue retaining the potentiality for growth can be made the site of the malignant action. In perusing this list it is immediately apparent that the agents are extremely diverse, ranging from simple inorganic chemicals through the more complex organic molecules to the very specific macromolecular entities, the viruses. Considering this range of agents and the variety of sites affected we come to the realization that cancer can be viewed most profitably as a family of diseases rather than a single entity. Despite this likelihood, cancer cells from all malignant states exhibit certain common characteristics which are presented in Table 2 and which usually are responsible for the course of the disease.

The most basic asset of the cancer cell is its reproductive advantage over its normal cell of origin, particularly when it has been thrust into a new extracellular environment. Without this attribute there could be no cancer problem. This does not mean that cancer cells are always the most rapidly growing cells in the body; to the contrary, certain cells from the crypts of the intestine or the bone marrow may actually reproduce just as fast or faster than tumor cells. However, as the normal cell migrates into a new cellular association or if the extracellular environment about it is changed for any reason such as with the alteration of the level of a particular hormone, it will adapt to this change with a curtailment of cell reproduction. On the other hand the malignant cell either adapts differently or fails to adapt, thereby permitting the continued production of progeny.

It is the research toward the elucidation of this essentially qualitative difference between the cancer cell and the normal cell which offers most hope for a solution to the cancer problem.

The Carcinogenic Process

Inferred in the above discussion is the conclusion that cancer cells breed true and that a part of the malignant transformation affording them a repro-

Table 1—Examples of the Variety of Carcinogens

Inorganic
Selenides
Chromates
Organic
Polycyclic aromatic hydrocarbons: (3, 4 benzpyrene)
Aromatic amine and aminoazo dyes: (2 acetylamino-fluorene and 4 dimethylaminoazobenzene)
Alkylating agents: (N-stearoyl ethylenimine)
Ethyl carbamate
Ethionine
Tannic acid
Viruses
Leukosis (fowl)
Sarcoma (fowl)
Kidney carcinoma (frog)
Papilloma (rabbit)
Mammary carcinoma (mouse)
Leukemia (mouse)
Polyoma (mouse, hamster)
Radiation
UV Light
Alpha-, beta-, gamma-, and x-ray

ductive advantage involves some change in genetic character or in the mechanism for expression of specific genetic factors. While this is not yet an established fact, studies on the mechanism of carcinogenesis support this general concept. The work of Boutwell, Rusch, and others has demonstrated that a very small amount of carcinogenic hydrocarbon such as dimethylbenzanthracene can produce in a single brief contact an irreversible or persistent change in the skin of the mouse. In itself, this change does not yield a cancer, but if provoked by a subsequent, delayed application of the proper promoting agent, a tumor will arise at this site.[1-3]

As an example of this situation Figures 1 and 2 present two groups of mice, both of which received a single application of 10 micrograms of dimethylbenzanthracene to initiate the carcinogenic process. The group of animals without tumors received no further treatment, whereas the tumor-bearing group shows the effect of a subsequent treatment with a promoting agent, croton oil. While the croton oil alone did not produce cancer it did "promote" the development of those tumors incited by a small initiating dose of the carcinogen, dimethylbenzanthracene.

Similarly in Figure 3 the promoting action of skin wounding on tumor formation is demonstrated. It is to be emphasized that we now know numerous promoting agents, some of which are common chemicals such as phenols, detergents, and surface active agents. Their action is, however, separate and distinct from that of the primary carcinogen.

Studies of this type demonstrate that the carcinogenic process can consist of multiple or sequential events. Thus it is quite possible to encounter carcinogenic stimuli which do not lead to the production of tumors or to encounter promoting factors without serious consequences. Unfortunately the opposite situation is also true; and since both such factors are becoming increasingly prevalent in our everyday lives it is necessary to elucidate further these relationships if we are to live with the minimum of hazard.

Table 2—Characteristics of the Cancer Cell

1. The Cancer Cell exhibits a reproductive advantage over the normal cell of origin.
2. The Cancer Cell exhibits a great potentiality for dissociating from one cellular environment and setting up a new growth at another site.
3. The Cancer Cell is endowed with a high capacity for change which leads to adaptation and selection toward greater malignancy.

Figure 1—The mice received a single application of 10 micrograms of dimethylbenzanthracene followed by one drop of benzene once a week for 12 weeks. Note that this sequence does not give rise to tumors. (Photo provided by Dr. R. K. Boutwell.)

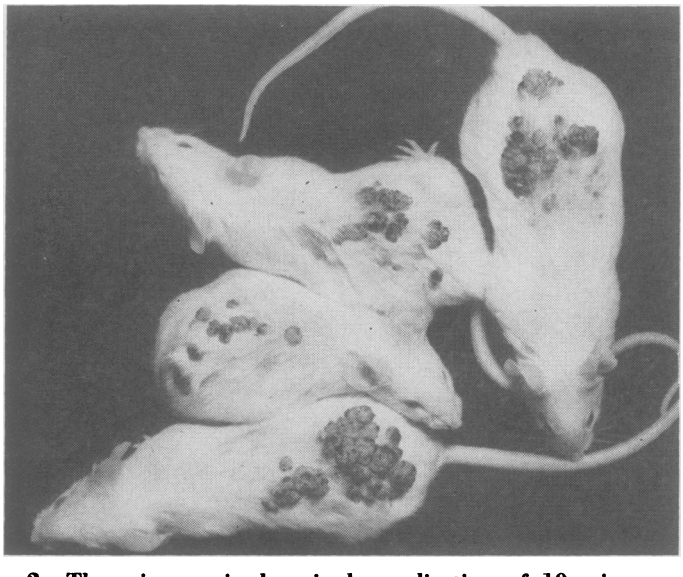


Figure 2—The mice received a single application of 10 micrograms of dimethylbenzanthracene followed at weekly intervals for 12 weeks with one drop of a 0.5 per cent solution of croton oil dissolved in benzene. Note the large numbers of tumors which have arisen on the backs of the mice. Croton oil alone did not produce tumors under these conditions. (Photo provided by Dr. R. K. Boutwell.)

A question which is always asked in studies on cancer production is: How does the carcinogen act? It is self-evident that knowledge of the molecular interactions of the carcinogenic agent with essential cellular constituents would go a long ways toward the elucidation of the mechanism of cancer production. In studies with the azo dye, dimethylaminoazobenzene, Miller and Miller have demonstrated a protein-bound form of this agent at the site of cancer production which correlates well with its carcinogenic potency and that of various derivatives.[4] Similarly protein-bound forms of other widely active carcinogens such as benzpyrene, dibenzanthracene, dimethylbenzanthracene, and acetylaminofluorene have been demonstrated.[4,5]

With the latter compound a special inquiry has been made as to the molecular form of the active carcinogen.[5] These studies have revealed a new biochemical process in which the nitrogen group of acetylaminofluorene is hydroxylated (i.e., N-hydroxylation) (Figure 4). This metabolite is more active as a carcinogen than the parent compound and is active at a greater number of sites (Figure 5). Since this is such a distinctive metabolite, further studies should be facilitated and should point out more clearly those interactions with cellular constituents which are of importance to the carcinogenic process.

While there are many unsolved as-

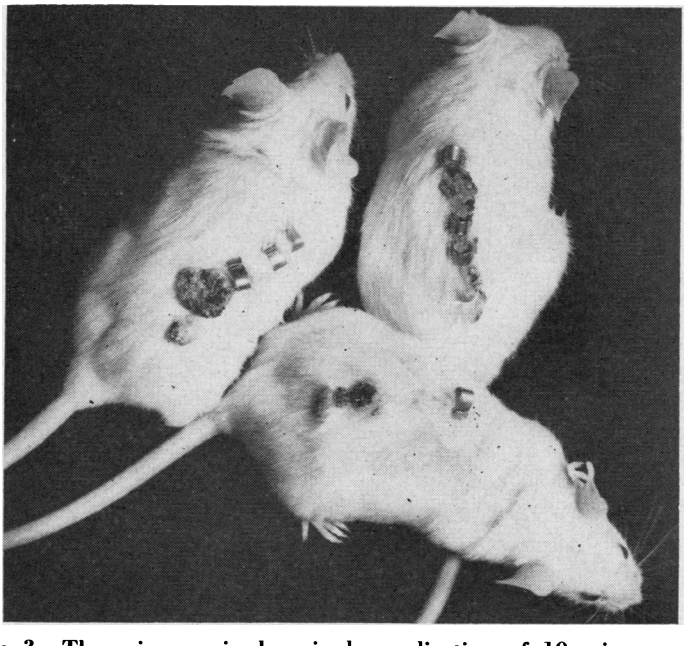
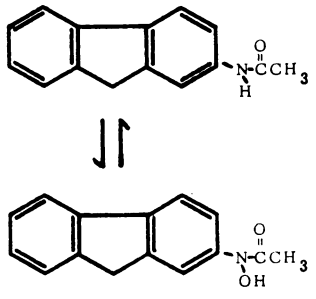


Figure 3—The mice received a single application of 10 micrograms of dimethylbenzanthracene followed at weekly intervals for five weeks with one drop of a 0.5 per cent solution of croton oil dissolved in benzene. The animals were then wounded by a skin incision which was allowed to heal with wound clips in place. Note the localization of the tumors along the incision line at 12 weeks. Very few tumors arose on the backs of mice receiving croton oil for five weeks, but not wounded. Wounding in the absence of the croton oil pretreatment was ineffective. (Photo provided by Dr. R. K. Boutwell.)

2-ACETYLAMINOFLUORENE



N-HYDROXY 2-ACETYLAMINOFLUORENE

Figure 4—The structural formulas for acetylaminofluorene and the N-hydroxylated derivative

pects of this work, it is apparent that the model systems are gradually yielding to the inquisition. It is expected that the information derived from these studies will be of great value in understanding the genesis of human cancer and in gaining control over it.

Viruses and Experimental Cancer

An exciting and rapidly expanding area of current cancer research concerns the role of viruses in cancer. As the result of new technological advances and the improvement of general tissue culture procedures, a number of animal tumors have now been shown to have a viral etiology. The best resolved tumor-virus systems include the Rous, polyoma, mouse leukemia, and the mouse mammary tumor virus systems. The infectious agents have been shown to represent both classes of viruses: those containing desoxyribonucleic acid and those containing ribonucleic acid as the hereditary material.

A major attribute of the viruses as carcinogens lies in their high efficiency in producing the malignant transformation. In the case of the Rous virus the infection of a fibroblast with a single particle transforms it within a few hours into a fully malignant sarcoma cell. This transformation is attended by a morphologic alteration of the cell which permits the recognition of the newly formed tumor cells.[6] Using these principles for an assay it has been possible to study the infection process in considerable detail. In Figure 6 a focus of virus infection on a monolayer of chick embryo fibroblasts is revealed as a pile-up of round, refractile cells.

From studies of the changes in morphology and growth patterns of the infected cells it is evident that the virus endows the host cell with new properties which must in some manner be related to the malignant change. Present research inquires into the nature of these changes. There are some indications that the relationship of the virus to the host cell is a very subtle one and possibly one involving a close association of both genomes.[7] Progress in this area awaits a better understanding of virus-host cell interaction in general, but with particular respect to the manner in which the virus assures its own duplication and the manner in which it influences the reproduction of host cell constituents.

Viruses as Causative Agents

With a significant number of different types of tumors in a variety of animals traced to a viral etiology it is only natural to inquire how extensive a role do viruses play in the causation of cancer. In animals there are many tumors in which, despite careful studies, it has not been possible to demonstrate a virus etiology. This is particularly true for chemically induced tumors. Accordingly it would appear that tumors in animals may be incited by a variety of agents of which a virus is a single example. However, simply observing some of the complexity of the existing

systems should prompt the maintenance of an open mind on this subject.

With respect to the role of viruses in human cancer it can only be stated at this time that there is no conclusive evidence indicting a virus as a causative agent for human cancer. However, many studies are in progress to test this possibility exhaustively; and much emphasis in these efforts is placed on acute leukemia, Hodgkin's disease, and certain intestinal malignancies in which the character of the clinical disease is suggestive of a viral process. A positive finding would have obvious implications for both the therapy and prevention of the particular malignant state.

The Cell Reproductive Cycle

In the discussion so far the cell has been dealt with mainly as the recipient of a carcinogenic stimulus; let us now consider briefly the cell response. As stated earlier, the cancer cell expresses a malignant transformation by the acquisition of a reproductive advantage over the normal cell. It is therefore of

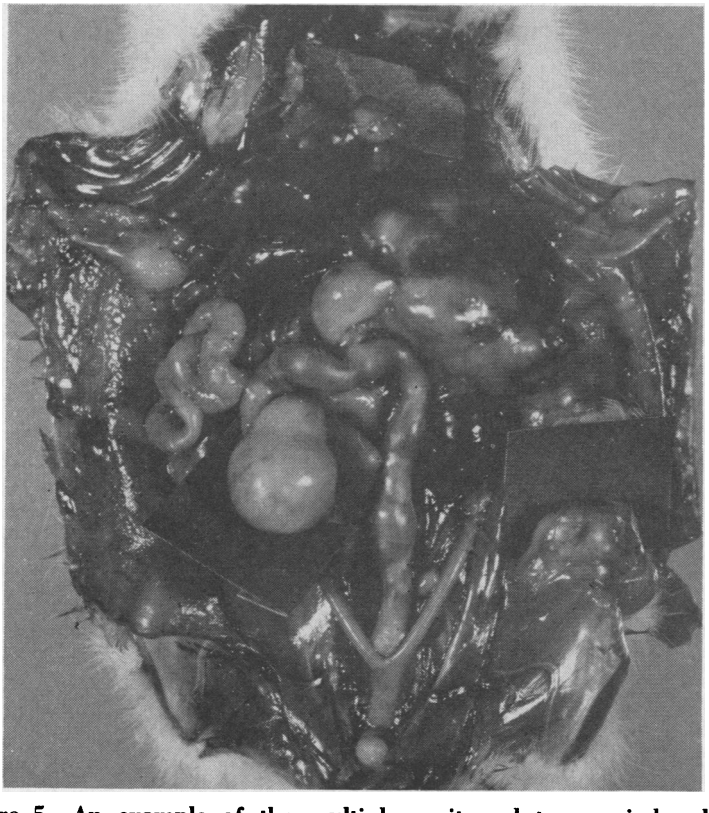


Figure 5—An example of the multiple peritoneal tumors induced by N-hydroxy-acetylaminofluorene. This rat received 6 mg of the carcinogen three times a week for three months at which time many tumors were evident. This agent also has been shown to produce tumors of the liver, ear duct, mammary gland, small intestine and the forestomach. It is more active as a carcinogen than the parent molecule (Figure 4). (Photo provided by Dr. J. A. Miller.)

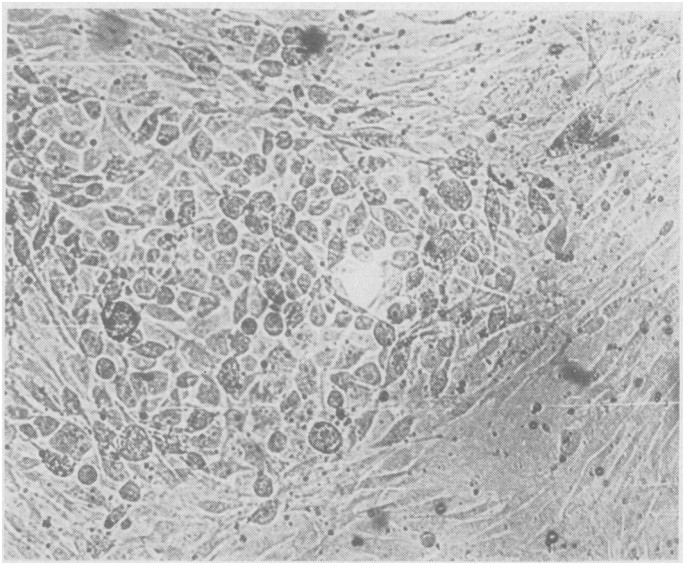
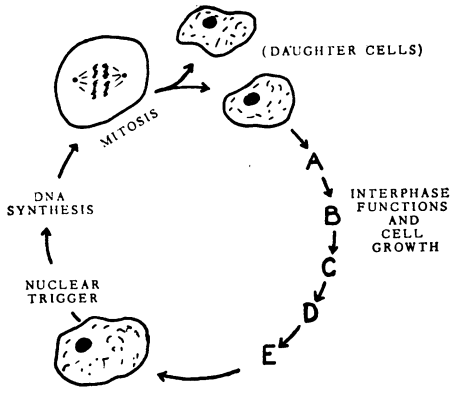


Figure 6—A foci of tumor cells resulting from the infection of a chick embryo fibroblast with a single infectious particle of Rous virus. Note the group of round refractile sarcoma cells which result from the growth of infected cells and the spread of the infection to adjacent cells. The normal fibroblasts are evident at the periphery of the monolayer. (Photo provided by Dr. H. M. Temin.)

great importance to elucidate some of the regulatory processes which can restrict the frequency at which cells replicate.

For such studies a cell can be considered to proceed around a cycle on the way to the generation of two new daughter cells; these in turn re-enter this same cycle as illustrated in Figure 7. In a tissue culture cell such as HeLa with a generation time of 24 hours, approximately 30 per cent of the time is spent in the process of synthesizing its nuclear apparatus and the carrying out of the mitotic process. The rest of the time (16 hours) is consumed in passing through the hypothetical steps indicated by A, B, C, D, and E. In the case of most normal cells in situ such as those present in the liver, kidney, lung, etc., the reproductive cycle may take weeks, months, or even years. However, once the nuclear synthesis has been initiated in these cells the time involved for the completion of this process is of a similar order to that observed in our fastest replicating animal cells. It is therefore indicated that the prolonged cycle of normal cells results from a hold up or extension of the interphase period represented by the letters A through E.

Unfortunately we know very little of the events in the interphase period which lead up to and trigger the synthesis of the nuclear apparatus. Studies in our laboratory have revealed that an important accomplishment of this period is the synthesis of a specific protein which is involved in converting the desoxyribonucleic acid of the chromosomes into a state which is competent for duplication of this genetic material. It is anticipated that further investigation of this area will elucidate the reasons for the all or none character



CELL CYCLE

Figure 7—A diagram of the reproductive cycle of a mammalian cell. Symbols A-E represent hypothetical stages or barriers which have to be overcome in the progress of the cell toward the initiation of a nuclear synthesis phase

of this process and point up the manner in which agents such as carcinogens, hormones, and viruses are able to modify this process.

It is suggested that investigations in this area are of the greatest importance to the design of chemotherapeutic agents against cancer; such agents might be expected to have a selective therapeutic effect characterized by a physiological regulation of cell growth rather than a generalized toxicity for all rapidly growing cells as do our present anticancer compounds.

In summary, it is highly encouraging to note the rapidity at which the various areas of fundamental research in cancer are developing. While the problem is as intricate as life itself, there is abundant evidence to encourage us in our conviction that it is yielding to the dissective approaches of modern biochemistry and biophysics.

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